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The purpose of this proposal was to elaborate on the viability of NIS-mediated genetically targeted radiotherapy as a possible novel therapeutic novel therapeutic intervention in human breast carcinoma. Problems encountered with transfection of SK-Br-3 forced other cell lines to be utilized in the development of stable NIS expressing clones while continuing to try other transfection methods. Stable NIS expressing clone was derived from MDA-MB-435 cell line. The ability of the clone to accumulate radioactivity was lost after several passage which may be due to epigenetically silencing. The NIS expressing clone was unable to accumulate radioactivity *in vitro* or *in vivo*. Real-time RT-PCR experiments are examining the detection of NIS expression after retinoic acid treatments. This treatment may turn on lactoperoxidase expression as well increasing the retention of radioactivity in the tumor. LPO treatment did increase retention of I-125 in Ad-NIS treated cells compared to Ad-NIS treatment alone. The acquisition of a pin-hole collimator enables mice bearing Ad-NIS treated tumors to be non-invasively imaged following radioactive administration. The imaging enables dosimetric calculation to be performed to determine the absorbed dose to the tumor. Correlations between the absorbed dose and therapeutic outcome can provide a possible prediction of tumor response.

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Introduction

Genetically targeted radiotherapy utilizes gene transfer to directly target tumors through intratumoral injections of a therapeutic gene, specifically the sodium iodide symporter (hNIS). The transduced gene confers the ability to accumulate and retain a radioisotope. The resulting radioisotope accumulation leads to the death of not only the transduced cell but also the surrounding cells through a process known as the 'bystander effect.'

In healthy thyroid cells, the sodium iodide symporter facilitates uptake and retention of iodine in the thyroid gland. Once iodide is transported into cells by NIS, it is catalyzed by thyroperoxidase (TPO). The iodination (organification) of tyrosine residues on thyroglobulin helps retain the iodide until the thyroid is stimulated to release thyroid hormone. In addition to iodide, the human sodium iodide symporter has an affinity for several halides and pseudohalides, enabling better imaging with ^{99m}Tc-pertechnetate and potential therapy with ^{188}Re-perrhenate. This ability to concentrate isotopes has been utilized clinically for years in thyroid imaging, treatment of hyperthyroidism, and for treating well-differentiated thyroid cancer. ^{131}I is at least partially responsible for the excellent 10-year survival rates of thyroid cancer.

Accumulation and retention of radioiodide can be imaged for real-time assessment of therapy in breast cancer patients. In many breast cancers, an increase in lactoperoxidase could enhance radioiodide retention to elicit a lethal effect on the tumor. Transduced hNIS provides a novel therapy that can be used to deliver a lethal radiation dose to the tumor yet spare the normal tissue. This therapy will be important in minimizing the current undesirable side effects and morbidity associated with current treatment modalities.

This summary will discuss problems currently encountered and report new technology introduced into the lab. The new methodology, modeling clinical technique, will increase data gathered from each experiment yet decrease animal numbers. This will provide an edge for pre-clinical testing requested by the FDA for this study to go forward.

Body

The aim of task 2 was to create stable NIS expressing SK-Br-3 clones and empty vector control cells. These cells would then be used to establish the required percentage of transfected cells to elicit a desired therapeutic effect. Unfortunately, problems were encountered using the lipofectamine kit manufactured by Qiagen Inc. (Valencia, CA). Several trials with this method resulted is high cellular cytotoxicity. After attempting transfection with other kits, cytotoxicity was reduced using Effectene Transfection Kit (Qiagen Inc, Valencia CA). Resultant clones are currently growing and will be screened for radioactive accumulation.

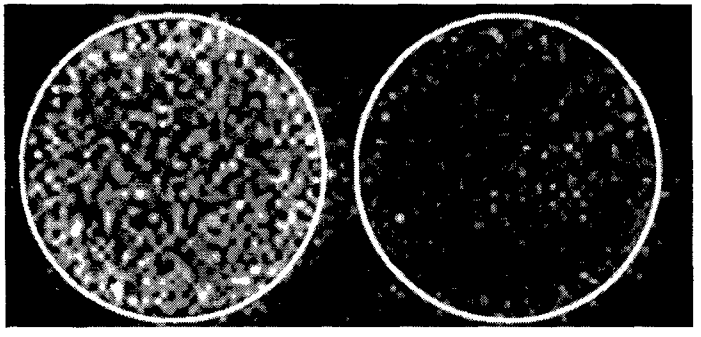

Figure 1. *In vivo* imaging with ^{99}mTc-pertechnetate. Clones were visualized and screened utilizing gamma camera scintigraphy. The clone was chosen according to radioactive accumulation. The clone on the left accumulated at higher levels than the cells on the right.

When transfection problems became apparent with the SK-Br-3 cells, other cell lines were employed to determine if it was a cell-line specific toxicity. One clone of MDA-MD-435 cells was thus created (Figure 1).

The radioactive accumulation from the original screening was observed at significantly higher levels contrasted to control cells. Although the clone was maintained in G418 media, after several passages its lost the ability to accumulate radioactivity (Figure 2).

This reduced radioactivity accumulation for the NIS-expressing clone was similar to that of the control clone. When imaged using the gamma camera, the NIS stable clone displayed no detected radionuclide accumulation over that of control (Figure 3, next page).

Athymic nude mice were then injected with the MDA-MB-435 stable NIS expressing clone. The clone was injected in the mouse's right flank and with the control clone injected on the contralateral flank. The resultant observation indicated the NIS stable expressing tumor detected no increased signal; the

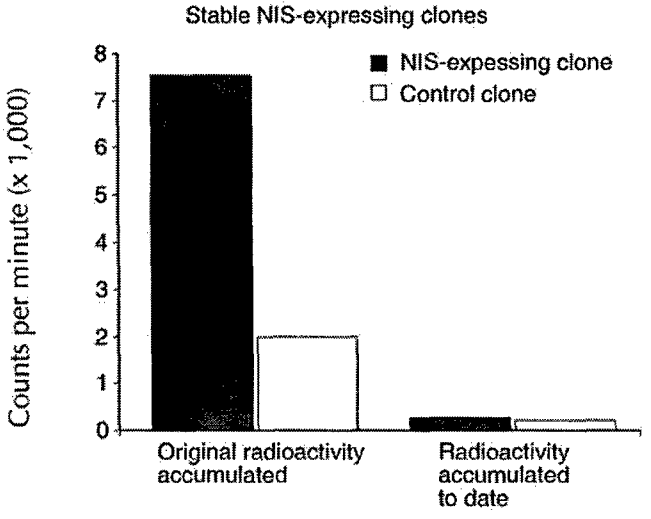

Figure 2. The original clone displayed higher radioactive accumulation compared to control. After several passage the clone lost the ability to accumulate radioactivity.

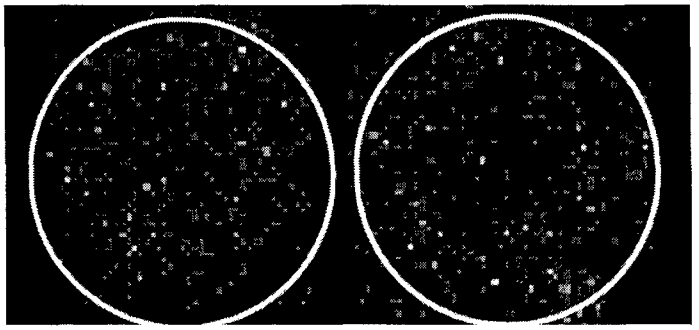

Figure 3. *In vivo* imaging of NIS expressing clone and the control clone. The ability to accumulate 99mTc-pertnetate is reduced to level of the control clone. Compare to Figure 1.

signal was similar to that of the control tumor. The thyroid, stomach and bladder where all visible (Figure 4).

A recent paper described this same phenomenon in stable NIS expressing clones. The investigators found NIS expression was silenced due to chromatin condensation and methylation. Treatment with a demethylating agent and a histone deactylase inhibitor reactivated the transgene.[1] The reversal implies epigenetic modulation of the sodium-iodide symporter.

Another report[2] cited treatment with retinoic acid (RA) could have similar effects with respect to increasing endogenous NIS expression. Currently, experiments are in progress to explore the effect RA has on NIS expression in breast cancer cell lines. With a newly acquired real-time RT-PCR machine (Abi 7000 sequence detector, Applied Bioscience, Foster City CA) our lab has quantitatively analyzed RNA expression levels of NIS. Data from preliminary experiments using RA are promising; showing detectable differences in NIS between the RA treated cells and untreated T47D.

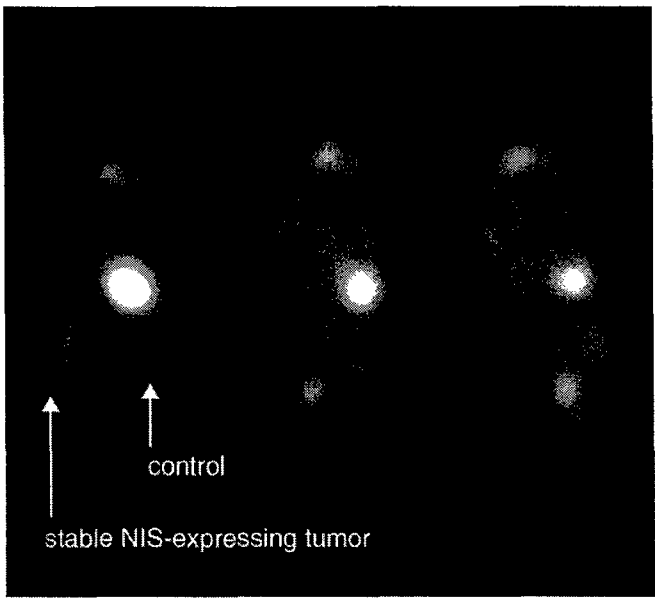

Figure 4. *In vivo* imaging of MDA-MB-435 stable NIS-expressing clones compared to control. The NIS stable expressing clone displayed no significant accumulation of ^{99}mTc-pertechnetate.

In future experiments utilizing retinoic acid, we will also examine the expression of lactoperoxidase (LPO) after RA treatment. Cells expressing both NIS (either endogenously or transfected with Ad-hNIS) and LPO will not only accumulate more radioactivity but retain that radioactivity for longer periods of time. This increase in retention should increase the bystander effect and thus the absorbed dose to the tumor. Both Zr-75-1 and T47D cells treated with Ad-hNIS, LPO, and glucose oxidase displayed a higher retention of I-125 contrasted to cells treated with Ad-NIS and I-125 alone.

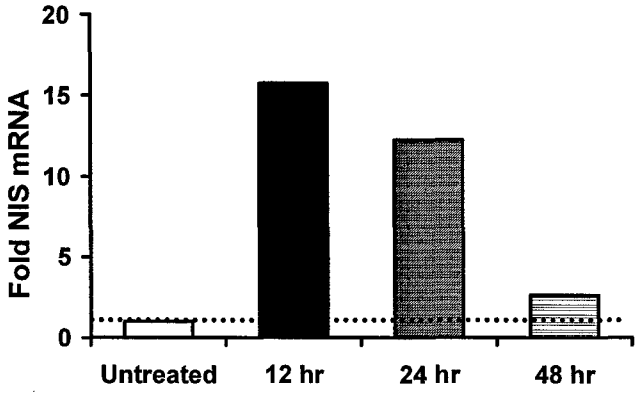

Figure 5. Increase in endogenous NIS expression following RA treatment detected using real-time RT-PCR. Cells treated with RA displayed a higher expression of NIS than untreated cells.

We proposed whole body autoradiography to determine biodistribution and dosimetry (Task 5). Recently, we have fitted our gamma camera with a pin-hole collimator to enable dosimetry calculation to be performed in the Ad-NIS/I-131 treated mouse. The development of this technology enabled our lab to determine the absorbed dose the tumor receives in addition to the dose to the normal tissue. The significant advantage of the pin-hole collimator contrasted to autoradiography is the negation of animal sacrifice. A pin-hole collimator does not require the mice to be euthanized prior to exposure like the phosphorimaging screen. This greatly impacts Task 1; we can inject the tumors with Ad-NIS or Ad-Bgl II, and (following the clinical protocol) forty-eight hours following intratumoral injection I-131 will be administered. The animals will be imaged non-invasively and dosimetric calculation will be performed using counts received from the images. The tumors will be monitored following the imaging. The absorbed dose calculated from the images can be correlated to the therapeutic outcome of the tumor. The ability to measure NIS activity with functional imaging suggests it may become possible to more accurately predict the tumor's response. This will reduce the animal numbers needed to perform both tasks while maximizing the data produced from one single experiment. The preliminary experiment withan athymic nude mouse bearing SK-Br-3 tumor treated with Ad-NIS/I-131 resulted in an absorbed dose of 100 rads to the tumor.

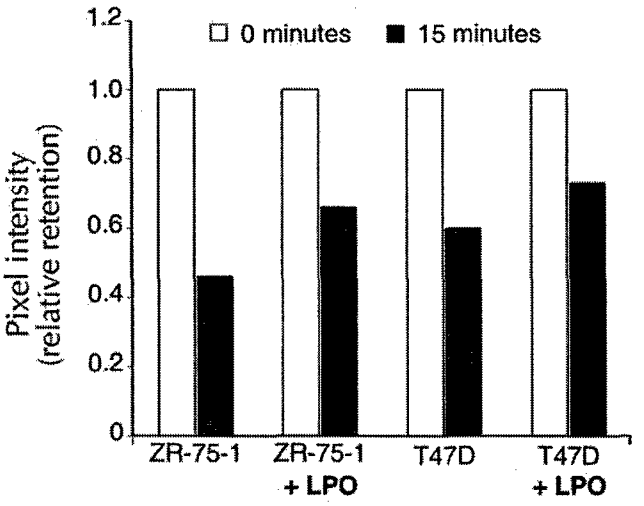

Figure 6. Cells treated with lactoperoxidase (LPO) and glucose oxidase were able to retain I-125 at higher levels than cells not treated with LPO.

Key Research Accomplishments

Creation of stable NIS expressing clones derived from MDA-MB-435 cells. Clones accumulated higher levels of radioactivity than the control. The expression, however, was increasingly lost after several cell passages. This unexpected silencing could be due to methylation or histone condensation. Methods to reactivate the transgene are being researched.

Retinoic acid. Experiments designed to increase NIS expression by using retinoic acid have resulted in the ability to quantitatively analyze expression levels and differences between treated and untreated cells. By establishing this method, further experiments examining the lactoperoxidase expression will be performed.

Clinically-relevant dosimetry. Dosimetric calculations using the pin-hole collimator fitted on the gamma camera enables non-invasive imaging and absorbed dose determination. This real-time dosimetry negates the need for animal sacrifice; thus, the long-term tumor response can be continually monitored. This advantage (contrasted to autoradiography) provides the correlation between absorbed dose and therapeutic outcome. This enables better treatment predictions for tumor response in later experiments. An added advantage is the reduction of the number of animals needed to perform the dosimetry, therapy experiments, and will ultimately increase the data received.

Reportable Outcomes

Data received from this grant has been accepted as a poster presentation at the 4th annual Era of Hope Breast Cancer Conference (Department of Defense Breast Cancer Research Program Meeting. Philadelphia PA).[3]

Conclusion

Stable NIS expressing clones accumulated radioactivity at higher levels than control. This accumulation was lost, however, after several passages and may be due to gene silencing. Treatment with RA is being examined to determine if NIS expression can be increased as well as inducing LPO expression. Lactoperoxidase expression may help in retention of radioiodide in NIS-expressing cells through organification of the iodide onto tyrosine residues. Dosimetric calculations are now performed from a pin-hole collimator. This non-invasive method mimics clinical dosimetry and enables the monitoring of tumor response to continue after radionuclide administration. Thus, correlations made between the absorbed dose calculated and the tumor response will better predict the outcome of later experiments. These methods correspond to clinical treatment modalities and will be easily translated to a phase I clinical trial.

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